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COMPLETE AND ISOENZYME CONTENT OF LACTATE DEHYDROGENASE IN PLASMA, LEUKOCYTES AND ERYTHROCYTES IN MYELOPROLIFERATIVE DISEASES

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COMPLETE AND ISOENZYME CONTENT OF LACTATE DEHYDROGENASE IN PLASMA, LEUKOCYTES AND ERYTHROCYTES IN MYELOPROLIFERATIVE DISEASES

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ABSTRACT. Examinations of the complete activity of LDH and its isoenzymes in the plasma and in myeloid cells revealed clear differences between healthy persons and patients with myeloproliferative diseases. Even within the latter group there were differences which can be used for a differential diagnosis. However, the long time which is required to isolate the cells, to represent the isoenzymes and to evaluate them will cause this method to be applied only in special laboratories or for special cases.

Lactate dehydrogenase (LDH) is one of the most important enzymes of the /229* glycolysis main chain. The changes in the LDH content of plasma or serum have led in the last decade to an increase in information available for clinical diagnostics as well as concerning hematological diseases. This is especially relevant to hemoblastoses.

Following the discovery of the heterogeneity of LDH, i.e. its isoenzymes, their physiological distribution was investigated by chromatographic and, more frequently, electrophoretic methods in plasma and tissues. As a rule, five isoenzyme fractions were found (LDH 1 to 5), which are depicted genetically as a kind of hybrid formation [1, 10] from two subunits, the so-called heart (H) type and the muscle (M) type, in the form of all conceivable tetrameric combinations (LDH 1 = HHHH, LDH 2 = HHHM, LDH 3 = HHMM, LDH 4 = HMMM, LDH 5 = MMMM). LDH 1 and, less so, LDH 2, migrate anodically with agar electrophoresis, whereas LDH 4 and, more so, LDH 5 migrate cathodically.

For blood diseases, apart from determination of total LDH and, above all, investigations of the isoenzyme in the serum, there is very little information available concerning the distribution in normal and morbid cells. The most interesting should be those determinations in normal and leukemic leucocytes which only became possible through appropriate cell separation processes. For

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*Numbers in the margin indicate pagination in the foreign text.

this reason, earlier investigations with not very pure cell isolations [9, 19] could not be completely confirmed by findings which were based on almost pure cell populations [4, 5, 12, 13].

All that is known about total LDH, and especially the distribution of /230
its isoenzyme in the case of normal persons and leukemia patients, can be briefly summarized [4, 5, 9, 12, 13, 19]:

1. Normal plasma, granulocytes, lymphocytes and erythrocytes may be clearly differentiated both in total LDH as well as in their isoenzyme prototype. Plasma: 5 bands, anodic; erythrocytes: 4 bands, anodic; granulocytes: 5 bands, cathodic.

2. Total LDH in plasma differs in the case of immature cell and mature cell myeloid leukemias in myeloid cells. This is, however, only true with immature cell leukoses.

3. Immature normal granulocytes coming from the bone marrow show an isoenzyme prototype differing from the promyelocytoid or myeloblastoid parablasts from the peripheral blood in the case of acute myeloid leukemia (AML).

4. Myeloid cells in the case of chronic myeloid leukemia (CML) should show a same isoenzyme prototype as normal granulocytes of the same stage of maturity.

5. Quite pure cell isolations are necessary for the investigations.

6. In addition to the relative values (%), the absolute ones were not always taken sufficiently into consideration.

These findings are essentially based on those of Rabinowitz and Dietz [13], although not always in agreement in detail, and caused us to initiate our own investigations with the following series of questions.

1. Are physiological LDH values in plasma, erythrocytes and granulocytes to be confirmed?

2. What changes of total LDH and its isoenzymes are there in the case of myeloproliferative diseases, chronic myeloid leukemia (CML), osteomyelofibrosis (OMF) and acute myeloid leukemia (AML) in plasma, in myeloid cells and in erythrocytes?

3. Is the determination of the isoenzyme prototype (rel. %) sufficient or must absolute values be determined?

4. Is a differential diagnostic utility present and can this be justified on an economic basis?

Material and Methodology

1. Investigations were carried out with 22 normal persons (blood donors), 10 patients with CML, 4 patients with OMF and one patient with AML.

2. Cell isolations:

a. The production of the erythrocyte sediment was done according to Richterich [14].

b. Normal and immature leukemic granulocytes in a number of ca. $3 \cdot 10^8$ were isolated according to Dioguardi, et al. [3, 5] with a purity between 90 and 98%. The remainder were lymphocytes and monocytes whereas erythrocytes and thrombocytes were completely lacking. The isolated granulocytes were phase-optically vital (movement, phagocytosis). The cell isolation according to Rabinowitz [11] was not always reproducible but was very costly from the time expenditure viewpoint.

3. Cell lysates:

a. Erythrocytes: H_2O-hemolysis, centrifuging out of cell stromata, adjusting the hemolysate to a hemoglobin concentration of 2.5 g/100 ml.

b. Granulocytes: lysis according to Yakulis, et al. [20] by carrying out high-speed freezing in acetone and dry ice three times, followed by homogenization.

c. Finally, preservation without loss of enzymes using temperatures under -70° C [13].

4. Determinations: /231

a. Total LDH: optical test according to Warburg and using the Fermognost instrument set of the VEB Arzneimittelwerk Dresden (Germed). Calculation as µmole NADH reaction/min/l or 10^{10} cells.

b. Isoenzyme determination: agar-gel-electrophoresis according to Wieme [18] (40 min, 190 V, 30 to 35 mA per microscope slide, diethylbarbiturate

sodium acetate buffer, pH 8.2), staining of the fractions with p-nitroblue tetrazolium chloride and phenazine methosulphate according to Dewey and Conklin [2]. Evaluation of the pherograms by photo-densitometric methods (Vitatron, Holland). Calculation of the relative values (in percent) and absolute values (using the total LDH determined at the same time).

Results

Total LDH Content

The lowest LDH activity was observed in the plasma of healthy persons, whereas the highest LDH activity was found in AML cases. The respective differences (Figure 1) can be contrasted statistically with the normal plasma although this could not be done with CML and OMF cases. Myeloid cells with CML showed a highly significant reduction in total LDH whereas those with OMF /232 showed an extreme increase.

Erythrocytes showed only slight, statistically not demonstrable differences in the case of myeloproliferative illnesses.

Isoenzymes

The agar-electrophoretic representation of the isoenzyme of granulocytes is shown as an example in Figure 2. The normal findings of plasma, granulocytes and erythrocytes are shown in Figure 3. It is possible to recognize the completely different isoenzyme distribution between granulocytes, on one hand, as well as erythrocytes and plasma, on the other hand. Fraction V, dominant in the granulocytes, does not occur in erythrocytes and only infrequently in plasma. Whereas the 4th and 5th bands dominate in granulocytes, we find the 1st and 2nd fraction dominant in erythrocytes and in plasma.

The average differences of absolute isoenzyme values for plasma (Figure 4) were significant with simple variance analyses using the H test up to fraction V. In addition, the differences between AML and CML were impressive. In the case of the latter, the increase of total LDH is caused especially through increases in the 2nd and 3rd isoenzyme fractions. Nevertheless, the 5th fraction remains unchanged. In myeloid cells (Figure 5) it is possible to find with myeloproliferative illnesses clear differences on a variance analytical basis within this group and in comparison with normal granulocytes.

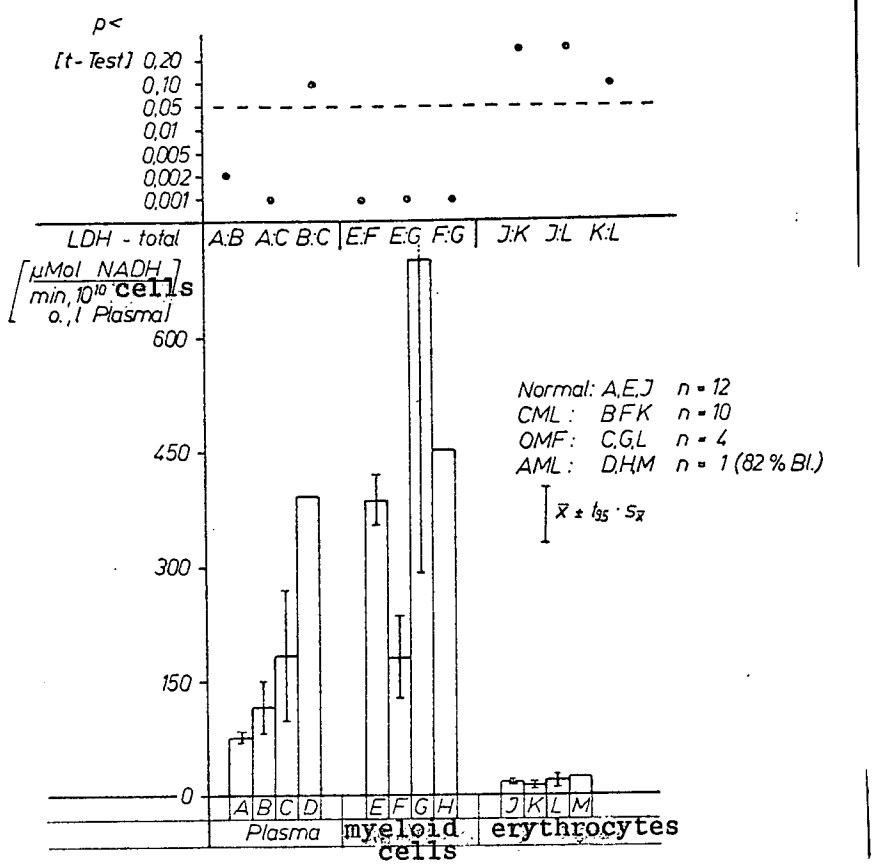

Figure 1. Total LDH content of plasma, myeloid and red blood cells of healthy persons and patients with myeloproliferative diseases.

Notations: CML = chronic myeloid leukemia, OMF = osteomyelofibrosis, AML = acute myeloid leukemia.

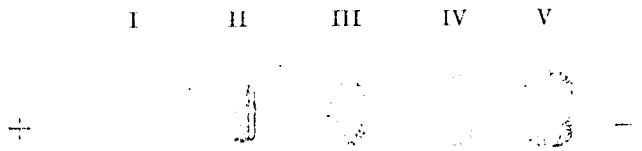

Figure 2. LDH isoenzyme distribution in normal granulocytes of the peripheral blood.

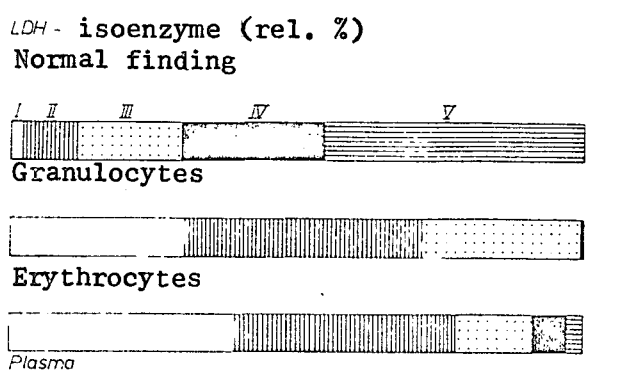

Figure 3. LDH isoenzyme prototype of normal granulocytes, erythrocytes and plasma.

With CML, there is a reduction of fractions 3 to 5 whereas with AML there /233 is an increase of bands 1 to 3 as well as a marked reduction of the 5th fraction just as in the case of CML.

A quite different isoenzyme picture can be observed with OMF. It most resembles the case with normal granulocytes although there is present a differential blood picture which is almost as strongly shifted to the left as in the case of CML, i.e. not the state of maturity of the myeloid cells but the qualitative differences determine the isoenzyme content of the granulocytes with myeloproliferative illnesses. All fractions are increased with OMF corresponding to the increase of total LDH. However, the second isoenzyme band is increased the most. The actual conditions can only be partially understood from the relative isoenzyme values. This can be seen from Table 1 which allows comparison of both values. Whereas the relative values with OMF essentially depict the changes correctly, these percentage figures with CML allow no clear judgment.

The erythrocyte isoenzymes show no clear deviations from the normal condition analagously to their total LDH content.

Discussion

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The following consists of answers to questions asked at the beginning:

1. The total LDH activity of normal granulocytes determined by us shows with 386.2 ± 53.8 µmol NADH/min/10^{10} cells a good agreement with the value of 371 ± 62 reported by Rabinowitz and Dietz [13]. Determinations of

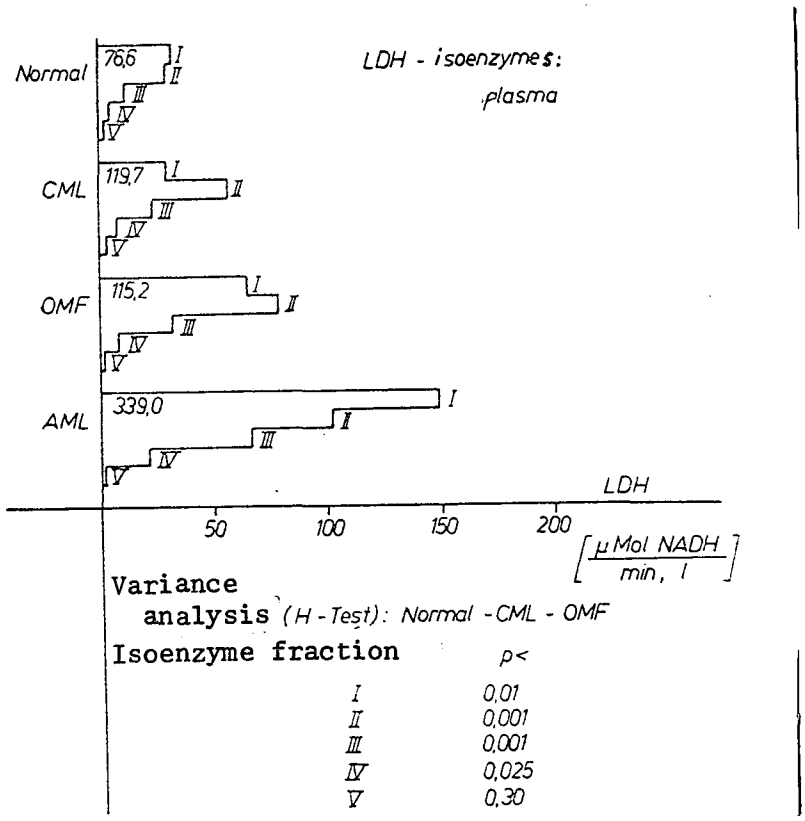

Figure 4. Absolute values of the LDH isoenzymes in plasma.

Notation: The surface of the step diagram corresponds to the total LDH activity and the value is given as a corrected number.

TABLE 1. LDH ISOENZYMES IN MYELOID CELLS AND PLASMA: COMPARISON BETWEEN RELATIVE AND ABSOLUTE VALUES (%)

	Normal	CML	OMF
Myeloid cells			
I	2,0% (7,52 ± 2,05)	8,3% (15,09 ± 7,76)	4,5% (31,5 ± 13,8)
II	9,6% (37,10 ± 9,36)	22,0% (39,84 ± 15,79)	21,7% (152,1 ± 92,4)
III	18,4% (71,72 ± 12,07)	26,2% (47,26 ± 16,89)	19,8% (139,0 ± 98,12)
IV	24,7% (95,10 ± 14,59)	19,7% (35,75 ± 11,08)	25,1% (176,1 ± 109,5)
V	45,3% (174,71 ± 35,9)	23,8% (42,96 ± 13,18)	28,9% (202,8 ± 127,8)
Plasma			
I	39,4% (30,2 ± 3,6)	23,3% (27,9 ± 6,77)	35,0% (65,0 ± 30,46)
II	38,3% (29,4 ± 4,22)	47,9% (57,24 ± 21,28)	42,7% (79,0 ± 42,34)
III	13,6% (10,4 ± 2,14)	19,7% (23,58 ± 10,14)	17,2% (31,8 ± 13,82)
IV	5,8% (4,4 ± 1,39)	6,3% (7,54 ± 3,08)	4,3% (8,0 ± 4,24)
V	2,9% (2,2 ± 0,75)	2,8% (3,4 ± 1,19)	0,8% (1,4 ± 1,42)

Notation: CML = chronic myeloid leukemia, OMF = osteomyelofibrosis. The absolute values are given in parentheses with confidence interval = $\bar{x} \pm t_{95} \cdot s_{\bar{x}}$

* Commas represent decimal points.

other researchers are located either significantly lower [4] or higher [9]. The values for plasma and erythrocytes likewise agree with those reported in the bibliography [13].

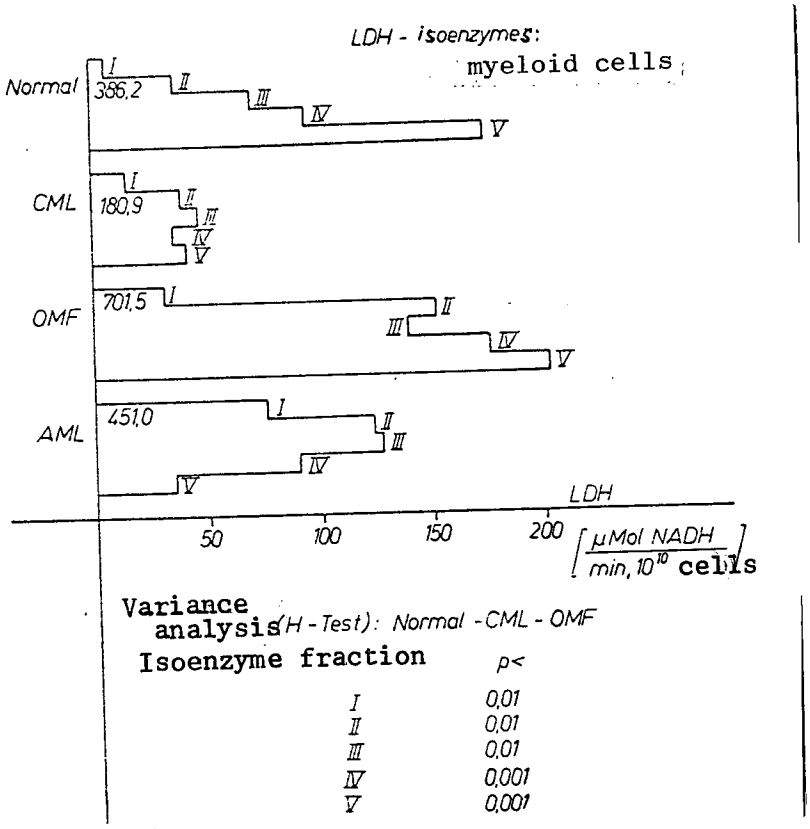

Figure 5. Absolute values of the LDH isoenzymes in myeloid cells.

Notation: The surface of the step diagram corresponds to the total LDH activity and the value is given as a corrected number.

The distribution of isoenzymes in normal granulocytes as determined with a pure cell suspension either electrophoretically [5, 13] or chromotographically [4] can be confirmed as preponderantly cathodic in nature with 5 fractions. Some more minor differences do exist, however, in the case of the somewhat different electrophoretic method.

There is an anodic isoenzyme prototype with only 4 fractions in the erythrocytes whereby the LDH 4 appears only as a trace. This finding corresponds to investigations which are already available [13]. Five isoenzymes were demonstrated in the plasma. The distribution is preponderantly anodic and, to be sure, having more than 90% enzyme activity in bands 1 to 3. This distribution confirms the findings of other researchers to the fullest extent [8, 16, 17].

2. In the case of myeloproliferative illnesses significant differences with respect to normal conditions have been demonstrated both in the plasma as well as in the myeloid cells. An increased total enzyme activity in the myeloid cells of the plasma of CML was found rather than a reduced one. The increased plasma activity has, on the other hand, frequently been noted [6, 7, 15, 19, 20] just as the increased LDH_2. Also, LDH_3 was observed at the same time to be increased although on not as frequent a basis.

Differing from previous reports [4, 5, 13], we found a highly significant statistical decrease in the total LDH activity of myeloid cells in the case of CML. This clearly affected the isoenzyme content but had less of an effect on the isoenzyme prototype.

3. Chiefly on the basis of the facts just described above, we find the relative values, i.e. the isoenzyme prototype unsatisfactory for detecting the actual changes (cf. also Table 1).

4. As can be seen from the results, a differential diagnostic significance may be attributed to the LDH isoenzyme content with myeloproliferative illnesses. This determination could be suitable for differentiating cytologically and clinically those aspects of a disease which have not yet been clearly determined (myeloproliferative syndrome) on an earlier basis. Of course, the considerable expense makes the method advantageous only for the larger special laboratories having sufficient trained personnel. Nevertheless, it is still conjectured as to whether the differential diagnostic gain justifies the employment of therapeutic means which are still in quite short supply today.

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